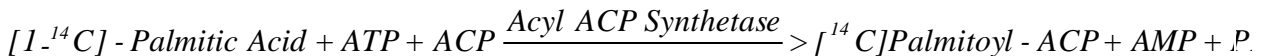


**Enzymatic Assay of
ACYL-ACYL CARRIER PROTEIN SYNTHETASE
(EC 6.2.1.20)**

PRINCIPLE:



Abbreviations:

ACP = Acyl Carrier Protein
ATP = Adenosine 5'-Triphosphate
AMP = Adenosine Monophosphate
P_i = Pyrophosphate

CONDITIONS: T = 37°C, pH = 8.0

METHOD: Radioactive

REAGENTS:

- A. 1000 mM Tris HCl buffer, pH 8.0 at 37°C
(Prepare 10 ml in deionized water using Trizma Base,
Prod. No. T-1503. Adjust to pH 8.0 at 37°C with
1 M HCl.)
- B. 4000 mM Lithium Chloride (LiCl)
(Prepare 5 ml in deionized water using Lithium
Chloride, Prod. No. L-8895.)
- C. 100 mM Adenosine 5'-Triphosphate (ATP)
(Prepare 5 ml in deionized water using Adenosine
5'-Triphosphate, Disodium Salt, Prod. No. A-2383.)
- D. 200 mM Magnesium Chloride (MgCl₂)
(Prepare 3 ml in deionized water using Magnesium
Chloride, Hexahydrate, Prod. No. M-0250.)
- E. 40 mM Dithiothreitol (DTT)
(Prepare 3 ml in deionized water using DL-
Dithiothreitol, Prod. No. D-0632. **PREPARE FRESH.**)

palmitic acid.

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PROCEDURE: (continued)

Pipette (**in microliters**) the following reagents into suitable microcentrifuge tubes:

	<u>Test</u>	<u>Blank</u>	<u>Back- ground</u>	<u>Total Counts</u>
Deionized Water	-----	20	30	30
Palmitic WS	20	20	20	20
Reagent G (ACP)	10	10	-----	-----
Reagent K (Enzyme)	20	-----	-----	-----

Mix by vortexing. Cap microcentrifuge tubes and incubate at 37°C for 20 minutes.

Transfer the Test, Blank and Background liquids to a 1 cm diameter disk of 3 mM Whatman filter paper suspended on a 2" x 22 gauge needle. Air dry at room temperature.

Transfer the entire vial contents of the Total Counts to a scintillation vial.

Rinse each filter paper disk twice with 12 ml each time of Reagent L (Chloroform:Methanol:Acetic Acid) for 5 minutes. Thoroughly dry the filters and place in scintillation vials containing 7.5 ml of Aquasol scintillation fluid and count with a suitable scintillation counter.

CALCULATIONS:

$$\text{nanomolar units/ml/ min} =$$

$$\frac{(CPM \text{ Test} - CPM \text{ Blank})}{(20)(.020)} \times \frac{1}{\text{Total Counts}} \times \frac{8.5 \text{ nanomoles } (1^{14}C) \text{ Palmitic Acid}}{RM}$$

RM = Reaction Mix
0.02 = Volume of Enzyme used
20 = Time of reaction in minutes

UNIT DEFINITION:

One unit will produce one nanomole of palmitoyl-acyl carrier protein from palmitic acid and acyl carrier protein per minute at pH 8.0 at 37°C.

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FINAL ASSAY CONCENTRATIONS:

In a 50 μ l microliter reaction mix, the final concentrations are 3.81 mM ATP, 76 mM tris, 304 mM lithium chloride, 7.6 mM magnesium chloride, 1.5 mM dithiothreitol, 0.01% acyl carrier protein, 8.5 nanomoles of 1^{14}C palmitic acid, 0.002 - 0.01 nanomolar unit of acyl-ACP synthetase.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock number.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.